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Endometrial and Decidual Stromal Cells Modulate Uterine ILC3 Function*in vitro*.

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Abstract:
Introduction: The decidualization of the endometrium is a prerequisite for the development of the placenta and thus for the maintenance of pregnancy. In this process, endometrial stroma cells (ESCs) undergo structural and functional changes and become decidual stromal cells (DSC). DSCs interact with the most important components of the placenta, including trophoblasts, endothelial cells and leukocytes, by the release of immune mediators. Particularly the immune modulatory capacity of ESCs and DSCs is still poorly understood. The aim of this work is to investigate the immunomodulating capacity of DSCs on the recently described lymphocyte population innate lymphoid cells type 3 (ILC3) with focus on the release of specific cytokines
Methods: ESCs were isolated from endometrial scratching and decidualized *in vitro*. The release of cytokines of ESCs and DSCs was determined by Bio-Plex Multiplex Immunoassay, qPCR and ELISA. ILC3s were isolated from *decidua basalis* from term births and the expression of receptors (IL-20R1, IL-22R1 and TGF-βR1-3) was determined by flow cytometry. ILC3 were stimulated *in vitro* with cytokines from DSCs (IL-6, IL-7, IL-24, SCF, TGF-β1, TGF-β2) in the presence of blocking antibodies (IL-6bAb and IL-7bAb) and ESC- and DSC-conditioned (ESC-CM/DSC-CM) medium for 48 h. Changes of proportion within the cell population of NCR^+ and NCR^-ILC3s were determined by flow cytometry. The data were analyzed by *t*-test and one-way ANOVA (Dunnett) and a p-value < 0.05 was considered as statistically significant.
Results: Stimulation of ILC3 with ESC- and DSC-CM, led to a decrease in the ILC3 cell fraction and the expression of CD69. The expression of ILC3-relevant cytokines IL-6, IL-7,

SCF, TGF-β1 and TGF-β2 was detected in the CM of both ESCs and DSCs. DSCs secreted higher levels of IL-7 and lower levels of IL-6 than ESCs. The stimulation of ILC3s with IL-6 and IL-7 led to significant increase of NCR^-ILC3, while IL-6 also induced a decrease of CD69. The blocking of IL-6 and IL-7 on CM had no effect on ILC3 phenotype. The treatment with TGF-β1 lead to decreased mRNA expression of TGF-βR3 on *in vitro* generated ILC3s.Decidual ILC3s expressed higher levels of the IL-24-receptor monomer IL-22R1 than the IL-20R1 monomer, and the levels were higher in NCR^+ILC3s than in NCR^-ILC3s. However, in contrast to previous reports, IL-24 was not detected in ESC- and DSC-CM.
Conclusion: Our results indicate that both ESCs and DSCs can influence uterine ILC3 biology by the release of soluble immune mediators.

Category (Complete): 11.4-Immune Function
Presentation Preference (Complete): Either Oral or Poster
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Early Career Investigator Award (Open to all Early Career Investigators who have not previously competed successfully as PD/PI for a substantial NIH independent research award). : True

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Please describe the translational relevance below : The interaction between ILC3s and endometrial stroll cells is important to understand mechanisms leading to endometriosis or early pregnancy loss.

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